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SHORT REPORT

The validity of capillary blood sampling in the determination of human growth hormone concentration during exercise in men

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Background: Studies measuring human growth hormone (hGH) in blood during exercise have mainly used venous sampling. The invasive nature of this procedure makes evaluation of hGH impossible in various exercise environments.

Objective: To determine whether capillary sampling could offer an alternative sampling method.

Methods: Capillary and venous blood samples were collected for determination of hGH at the end of each exercise stage during an incremental exercise test in 16 male club level competitive cyclists (mean (SD) age 30.8 (8.0) years, body mass 72.2 (7.1) kg, body fat 12.9 (3.5)%, peak oxygen consumption 4.18 (0.46) l·min⁻¹). Linear regression, from a plot of venous v capillary blood hGH concentration, showed a correlation coefficient of $r=0.986$ ($p<0.001$). When geometric means and log transformations were used, a coefficient of variation of 14.2% was demonstrated between venous and capillary flow for hGH concentration. The mean ratio limits of agreement were 0.62 (1.72)—that is, 95% of the ratios were contained between 0.36 and 1.07, with a mean of 0.62.

Conclusions: Capillary blood sampling is an acceptable alternative to venous sampling for determining hGH concentration during rest and exercise. Sample sites should not be used interchangeably: one site should be chosen and its use standardised.

Human growth hormone (hGH) is produced throughout life, the highest values being measured during puberty. It is released from the anterior pituitary gland in response to various stimuli, the most powerful of which are sleep and exercise. In general, hGH secretion follows a circadian rhythm and it is secreted in 6–12 discrete pulses per day, with the largest pulse approximately one hour after the onset of night-time sleep.

There have been growing numbers of studies examining the exercise induced hGH response. This response has been studied during endurance, resistance, and high intensity exercise.^{1–3} As well as the more obvious and recognised effects on growth (for example, on bone, visceral tissue, and muscle), growth hormone is also believed to affect the way in which the body uses fat and carbohydrate for energy during exercise. Specifically, hGH promotes the use of free fatty acids (FFA) as an energy substrate by inhibiting glycolysis and by mobilisation of FFA from triacylglycerol stores.^{4 5}

In general, hGH has been, and continues to be, measured during exercise in venous outflow, principally from an antecubital vein. In the exercise sciences there has been

considerable debate over the use of different sampling sites for measurement of changes in the concentration of analytes resulting from exercise (particularly blood lactate).^{6–9} Sample sites that have previously been used include the antecubital vein, the anterior ulnar vein, or the femoral vein for venous blood, the brachial artery or the femoral artery for arterial sampling, and the fingertip, toe, or earlobe for capillary sampling.

The invasive nature of venous sampling procedures renders the evaluation of growth hormone impossible in a variety of exercise environments. There is little doubt that an easier method of sample collection—that is, one that is less invasive and quicker to achieve at each sample point—would be advantageous and could help the field progress. The use of the less invasive capillary sampling methodology would therefore be more acceptable, but there is a dearth of reports on the use of capillary sampling, let alone its validity. Our aim in this study was therefore to establish the validity of measuring hGH concentrations in capillary blood as a more practical and cost-effective alternative to venous blood sampling.

METHODS

Following approval by the Harrow ethics committee of the Northwick Park and St Mark's Hospital Trust, 16 male club level competitive cyclists (mean (SD) age 30.8 (8.0) years, body mass 72.2 (7.1) kg, body fat 12.9 (3.5)%, and peak oxygen consumption 4.18 (0.46) l·min⁻¹) provided written, informed consent before participating in the study. All participants were engaged in regular cycle training and competition and were fit and healthy as determined by medical history. None of the volunteers had a history of pituitary, cardiorespiratory, renal, hepatic, or metabolic disease. Participants were non-smokers and refrained from exercise for at least 24 hours before each evaluation. Before testing, subjects had their body composition assessed using height and body mass (Avery Berkel, Birmingham) and body fat estimation from the sum of four skinfold sites.¹⁰

Testing protocol

Participants completed a continuous incremental cycling test on an SRM Ergoe cycle ergometer (Schoberer Rad Messtechnik, Fuchsend, Germany) to volitional exhaustion. The protocol consisted of six four-minute submaximal efforts followed by a seventh and final four-minute maximal effort. During the final effort subjects were instructed to attempt to cover the greatest distance possible while maintaining a relatively stable power output.

Throughout the exercise test, expiratory gases (Oxycon Alpha, Jaeger-Mijnhardt, Groningen, Netherlands) and heart

Abbreviations: FFA, free fatty acids; hGH, human growth hormone; IRMA, immunoradiometric assay

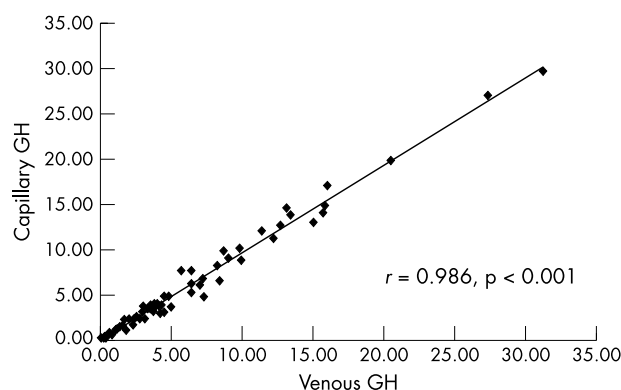


Figure 1 Linear regression plot for venous v capillary blood determinations of human growth hormone (GH) concentration (94 data points).

rate (Polar Vantage, Polar Electro Oy, Kempele, Finland) were measured continuously. Capillary (earlobe) blood lactate concentration was analysed as a single measurement at the end of each exercise grade using a GM-7 enzymatic analyser (Analox Instruments, Hammersmith, London, UK).

Blood samples for growth hormone measurement were collected during the last 45 seconds of each exercise stage simultaneously from an indwelling cannula inserted into a forearm vein and from the ear lobe, for capillary determination. Venous and capillary blood samples for hGH analyses were collected at -10, 19, 27, 35, 43, 53, 63, and 73 minutes—that is, two samples (one capillary and one venous) 10 minutes before exercise and after stages 1, 3, 5, and 7 and at 10, 20, and 30 minutes after the cessation of exercise. Saline was used to flush and so maintain the patency of the cannula before each venous sample collection.

Capillary samples were collected in 300 µl serum Microvettes (CB 300 LH, Sarstedt, Nümbrecht, Germany) placed on ice and spun in a microcentrifuge (Analox, Hammersmith, London, UK) within 60 minutes. Supernatant from capillary samples was then transferred to labelled Eppendorf tubes and frozen at -20°C for subsequent analysis of hGH concentration. Venous samples were collected into serum gel vacutainers, placed on ice, and spun in a refrigerated centrifuge. Supernatant from venous samples was transferred to labelled Eppendorf tubes and frozen at -20°C for subsequent analysis.

hGH analysis

Samples were assayed for hGH employing the coated-tube immunoradiometric assay (IRMA) kit produced by DSL

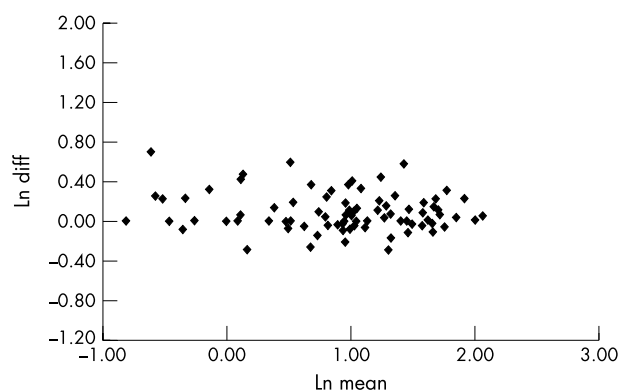


Figure 2 Log-log Bland-Altman plot for agreement between venous and capillary blood human growth hormone concentration.

(DSL-1900 ACTIVE, Diagnostic Systems Laboratories Inc, Webster, Texas, USA). This can deal with small sample sizes of 50 µl for analysis of hGH concentrations. In this case hGH is used as an antigen to produce antibodies in some other animal species, such as rabbit. These antibodies are then highly specific to hGH. The antibodies are added to a reaction vessel containing the serum hGH samples and radiolabel (in this case ^{125}I). As a result, this type of assay is sometimes referred to as a “sandwich assay” because the analyte is sandwiched between solid phase antigen and antibody–radiolabel complex.

A solid phase support is used for the reaction to take place on. In this particular assay the solid phase support is provided by cellulose particles. Cellulose is a stable polymer of glucose and reactions may take place in aqueous solutions (as in this case), at a pH of 3–10, and in organic solvents including dimethylformamide, dimethylsulphide, acetone, and dioxane.¹¹

Suspensions of cellulose have the advantage of a large surface area for immunoreagent or antigen binding. Efficient mixing during incubation with the analyte allows rapid and sensitive assays to be carried out. Cellulose particles are often used in immunoassays where the bound and free fractions are separated by centrifugation and washing,¹² as in this case.

The DSL assay procedure employs a two site IRMA in which the analyte to be measured is sandwiched between two antibodies. The first antibody is immobilised to the inside walls of the tubes, the second is radiolabelled for detection. The analyte present in the unknowns, standards, and controls is bound by both of the antibodies to form a sandwich complex. Unbound materials are removed by decanting and washing the tubes. The amount of bound hGH is directly proportional to the hGH present in the samples. The radioactivity reflecting the amount of bound hGH was determined by a 1261 Multigamma on-line counter (Wallac Oy, Turku, Finland).

Calibration and reliability

A standard calibration curve is produced by using known growth hormone concentration standards produced by the supplier of the IRMA kit. These standards undergo the same process detailed previously and are then run through the gamma counter.

The theoretical sensitivity, or minimum detection limit, calculated by the interpolation of the mean + 2SD of 10 replicates of the 0 µg·l⁻¹ hGH standards is 0.01 µg·l⁻¹. The intra-assay precision was determined from the mean of 12 replicates with three human serum samples. For 12 replicates each, sample 1 was 2.91 (0.09) µg·l⁻¹ with a coefficient of variation (CV) of 3.1%, sample 2 was 5.58 (0.22) µg·l⁻¹ with a CV of 3.9%, and sample 3 was 25.84 (1.40) µg·l⁻¹ with a CV of 5.4% (values are mean (SD)).

Statistical analysis

A linear regression plot and correlation coefficient is used to assess the strength of the relation between hGH concentration in venous and capillary blood. However, as correlation does not provide information on the agreement between the two measures, limits of agreement are used.¹³ As Bland and Altman pointed out,¹³ it is very unlikely that different methods, or in this case sample sites, will agree exactly by giving the identical result for all individuals. The point here is to assess by how much the concentration of hGH in capillary blood will agree with that in venous blood. Limits of agreement were felt to be the best method of distinguishing between two measurements and so this is the ideal statistical method in this instance, where determination of the agreement between capillary and

Table 1 Concentrations of human growth hormone and blood lactate

Sample time (min)	hGHven ($\mu\text{g}\cdot\text{l}^{-1}$)	hGHcap ($\mu\text{g}\cdot\text{l}^{-1}$)	Lactate ($\text{mmol}\cdot\text{l}^{-1}$)
-10	0.56 (0.85)	0.51 (0.69)	
19	1.03 (1.33)	1.09 (1.46)	1.31 (0.33)
23			1.51 (0.34)
27	2.21 (1.74)	1.89 (1.45)	1.81 (0.49)
31			2.44 (0.85)
35	5.66 (5.27)	5.03 (5.03)	3.58 (1.53)
39			5.27 (2.53)
43	15.98 (15.05)	12.63 (10.21)	8.45 (2.74)
53	15.98 (15.05)	9.11 (5.23)	
63	9.70 (5.48)	6.87 (5.33)	
73	6.43 (7.39)	6.52 (7.59)	

Values are mean (SD).

hGHcap, growth hormone in capillary blood; hGHven, growth hormone in venous blood.

venous samples is required. As the data demonstrate heteroscedasticity, we used geometric means and standard deviations calculated using log transformations to determine the coefficient of variation across three growth hormone ranges, 0–5, 5–10, and 10–15 $\mu\text{g}\cdot\text{l}^{-1}$. Ratio limits of agreement were also calculated.

RESULTS

A significant correlation coefficient ($r = 0.986$, $p < 0.001$) was observed between venous and capillary samples analysed for hGH concentration (fig 1). Coefficients of variation of 20.5% for 0–5 $\mu\text{g}\cdot\text{l}^{-1}$, 20.2% for 5–10 $\mu\text{g}\cdot\text{l}^{-1}$, and 12.1% for 10–15 $\mu\text{g}\cdot\text{l}^{-1}$ were observed, a coefficient of variation of 14.2% across the range of hGH measurement. The mean ratio limits of agreement were 0.62 (1.72)—that is, 95% of the ratios were contained between 0.36 and 1.07, with a mean of 0.62; the log-log Bland–Altman plot can be seen in fig 2. Table 1 provides mean (SD) values for venous and capillary hGH concentrations and for blood lactate at various time points before and during exercise.

DISCUSSION

Our study is the first to examine the validity of capillary sampling for determining blood hGH concentration. Our findings show that there is very good agreement between capillary and venous blood hGH concentration across a range of exercise intensities. A correlation coefficient of $r = 0.986$ ($p < 0.001$) was observed between venous and capillary samples, indicating that in 97% of cases capillary hGH concentration can predict the concentration in the venous outflow. A coefficient of variation across the range of measured hGH values of 14.2% was deemed acceptable. Hence, both good agreement (as illustrated by mean ratio limits of agreement of 0.36 to 1.07) and good strength of relation (as illustrated by an r value of 0.986) were demonstrated.

The problems of interfering with the movement of limbs during exercise and the more invasive nature of venous sampling often restricts the range of situations that will allow measurement of blood borne analytes. These results are thus of particular importance when investigating populations where there are concerns over the use of the more invasive venous sampling techniques during exercise—for example, in children, pregnant women, disabled individuals, and elite athletes. In addition, with capillary sampling reduced ethical concerns are apparent. Ethical concerns associated with venous sampling in elite athletes are related to the fact that there are increased risks of infection and bruising, which could limit the individual's ability to earn a living and affect their quality of life.

Even with such a good correlation and limits of agreement, as with lactate it is best to standardise the sample

site used. Accordingly, if blood is collected from a capillary site on one occasion, if subsequent comparison is to be made after a retest, then capillary sampling should again be used.

The methodological difficulties associated with simultaneous collection of an adequate volume of venous and capillary samples (that is, enough capillary blood for assay analysis) in 45 seconds highlights the need for repeated practice. Considerable expertise is required to collect venous and capillary samples within 45 seconds. We therefore recommend adequate practice in learning these skills to guarantee collection of the required volume within 60 seconds, the time after which a return to resting conditions is believed to begin.^{14 15} This is important where discontinuous exercise protocols are required, for example earlobe sampling using a treadmill or rowing ergometer. Difficulties encountered in sample collection include those resulting from peripheral vasoconstriction, which often accompanies exercise and affects both capillary and venous sites, coupled with blood coagulation in the cannulation set despite regular saline flushing. In addition, excessive squeezing of the capillary site or vacuum pressure used at the venous site will increase the risk of lysis of the red blood cells, and this can interfere with hGH measurement by the assay. Table 1 illustrates the large variation seen in the measurement of hGH regardless of sample site but this does not detract from the viability of capillary sampling.

In conclusion, capillary sampling for the determination of hGH concentration in blood before, during, and after exercise is an acceptable alternative to venous sampling.

Take home message

- Much is known on the comparison of blood lactate collected at different sites, but only one previous study has measured hGH in capillary blood. No information on this method or any attempt to validate it has been published. Therefore, to date, nothing further is known on the use of capillary hGH measurement in exercise.
- Capillary sampling provides hGH researchers with a cheaper viable alternative to venous sampling and therefore increases the range of circumstances, including exercise, in which it can be measured. Where ethical concerns are raised, this enables new populations to become accessible for research, providing opportunities for assessing hGH in exercise settings never before assessed.

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Authors' affiliations

R J Godfrey, Sport Sciences, Brunel University, Uxbridge, UK

G Whyte, BOMC, Northwick Park, Harrow, UK

J McCarthy, St Martin's College, Lancaster, UK

A Nevill, School of Sport, Performing Arts and Leisure, Wolverhampton University

A Head, School of Engineering, Cranfield University of Technology, Cranfield, Bedfordshire, UK

Correspondence to: R J Godfrey, Brunel University, Uxbridge, Middlesex UB8 3PH, UK; richard.godfrey@brunel.ac.uk

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